Section 1.0

1.0 Test Methodology

1.1 Comments regarding *in vitro* methods and test interpretation:

The agency states that certain products may require modification of the testing procedures and that alternate methods may be used but must be submitted as a petition and must demonstrate results of "equivalent accuracy". Since the existing test methods are of questionable value, we are of the opinion that *most* products will require alternative methods of testing and that the currently proposed *in vitro* test methods be listed as testing options rather than as testing requirements. Until such time as there is a reliable standardized *in vitro* assay for antiseptic activity, we feel the agency should use caution in the interpretation of *in vitro* test data, particularly MIC tests for determining the spectrum of activity of an antiseptic agent, and should place a greater emphasis on *in vivo* test data or on time kill studies

MIC testing:

Although Methicillin (MIC) data is extremely useful to determine the efficacy of antibiotics, which frequently cannot be administered at high enough doses to kill bacteria and yet be non-toxic, that is not the case with most antiseptics. Antiseptics often are used at a hundred- or even thousand-fold MIC levels. MIC testing has been widely demonstrated to inadequately correlate to the efficacy of antiseptics on skin.[1, 2, 3] For example, the large differences in MIC of chlorhexidine against gram-negative and gram-positive bacteria do not correlate with data obtained using seeded skin which indicated that the gram-negative bacteria were reduced more than the gram-positive bacteria.[1]

MIC tests are also only marginally useful in characterizing products that contain alcohol as a vehicle and/or one of a combination of active ingredients. When the active ingredient is water-soluble, the alcohol vehicle or combination active is virtually certain to be diluted to an inactive concentration during MIC determination thus having little or no effect on the MIC of the "active ingredient." Therefore, the MIC determination would not detect the significant contribution of alcohol to the spectrum of the final product as formulated for use.

Time Kill Testing:

Although more useful than MIC tests, especially for alcohol-containing products, time kill studies also can be unreliable indicators of antiseptic efficacy since most are done with bacteria in suspension which does not adequately reflect the localization of bacteria on skin squames and as clumps or microcolonies. [1] Time kill studies done in suspension usually indicate that bacteria are eradicated in as little as 15 seconds, which does not reflect the length of time it takes to kill those same bacteria on skin.[1,4] Indeed, in spite of the apparently short time it takes povidone-iodine to kill bacteria, there is clinical evidence that tincture of iodine, which kills even more rapidly, is more effective at reducing contamination of blood cultures, especially when contact times are short, something that would not

be apparent based on MIC testing.[1] Even the ASTM recognizes the superior efficacy of alcohol at removing transient organisms as evidenced by its recommendation that alcohol disinfection be used to ensure complete decontamination of the hands when testing an antiseptic hand wash, a fact that would not be revealed by MIC test, but would be apparent in a time kill study [333.470 (b)(2)(iii)(C)(3)].

1.2 Determine the *in vitro* antimicrobial spectrum [31444]:

3M supports the CTFA/SDA recommended list in the Healthcare Continuum Model as representative of the most clinically relevant organisms found in a healthcare setting. The number of test organisms needed (50 isolates of each of 21 species) is excessive. Testing this number of isolates for many products entails over 1000 tests for the active ingredient(s), vehicle and the final formulation, and is prohibitively expensive. Although there is some difference in the susceptibility of different species to various antiseptics, there is very little significant difference within a given species and even those differences in MICs that have been described do not correspond to a difference in clinical efficacy of the antiseptic.[6,7,8,9] The CTFA/SDA representatives have proposed to the agency that four clinical isolates (in addition to a designated ATCC strain) of 27 bacterial species which represent both normal flora and cutaneous pathogens, be tested for the active ingredient, and that a single ATCC strain of each species be used to test the final formulation for purposes of comparison. The list of proposed strains does not differ significantly from those test organisms listed in the amended TFM

1.3 Comments on ASTM methods referenced [333.470(a)(2)(i), (ii) and (iii)]

Within the TFM method descriptions the TFM states "The procedure to be used is a *modification* of the standard testing procedure for the evaluation of ... published by ASTM", and references the method, ASTM number (i.e. E1115 for surgical scrub) and Annual Book of Standards volume 11.04 for the three indications of surgical scrub, healthcare personnel handwash and preoperative prep. We request that the word *modification* be removed, and that the most current ASTM methods be required for testing. No particular volume of the Annual Book of Standards would be referenced. This new wording would be:

> The procedure to be used is a the standard testing procedure for the evaluation of ... published by ASTM", and references the method, ASTM number (i.e. E1115 for surgical scrub)

1.4 Comments regarding neutralization in Surgical Scrub, and Healthcare Personnel handwash methods [333.470(a)(2)(i) and (ii)]

We propose that the agency require neutralizers in all sampling solutions and that the most current ASTM methods be referenced for use. The methods described in the TFM for testing do not include neutralization until the very last sampling. We

have found that including neutralizer in the sampling solution, as well as in the agar plates, has a profound effect (>1 log) on both the immediate log reduction and the persistence of activity of certain active ingredients, especially those that are inherently difficult to neutralize. The most current ASTM methods for these indications now require neutralizers in the sampling solution for every sampling.